DETECTION AND PARTIAL CHARACTERIZATION OF TWO NEW POTEXVIRUSES INFECTING YAMS (*DIOSCOREA* SPP.)

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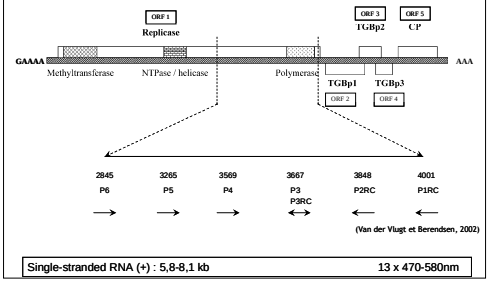
Virus diseases are one of the most important problems in yam (*Dioscorea* spp.) culture by reducing yield and tuber quality, and by causing the lost of sensitive varieties. Until now, different viruses such as poty-, potex-, badna- and cucumovirus, have been reported to infect yams (Brunt *et al.*, 1989). Among them, *Dioscorea latent virus* (DLV) was the only potexvirus found in medicinal yams *D. floribunda* and *D. composita* at Porto Rico (Phillips *et al.*, 1986). But, until now, no molecular characterization was done on this potexvirus and specific detection tools were not available.

In order to detect DLV during quarantine procedures at CIRAD, Filloux and Girard (2006) used potexvirus-specific PCR test and then reported the detection of several putative potexviruses on edible yams. We describe here the first partial biological and molecular characterization of two new putative potexviruses infecting yams detected by this way.

Fig 1: Schematic representation of the organization of the genome of Potexviruses



Materials and methods

- One hundred and ninety three worldwide yam accessions (Caribbean islands, South America, Madagascar, Pacific islands, Central and West Africa), including 122 *Dioscorea alata*, 55 *D. rotundata*, 13 *D. nummularia* and 3 *D. trifida* were screened for potexviruses.
- One step RT-PCR tests using potexvirus-specific degenerate primers (Potex1RC/Potex5) amplifying the C-terminal region of the viral replicase (van der Vlugt and Berendsen, 2002) were carried out (Fig.1).
- The 737 bp amplified products were single-pass double stranded sequenced and were BLAST searched against the GenBank database.

Results

- Four positive plants were detected, including 1 *D. rotundata* from Guadeloupe Island (FWI) and 3 *D. nummularia* from Vanuatu (Fig. 2).
- The 3 *D. nummularia* isolates were each other very similar at the nucleotide level (88.5-89.6% identity) (Fig. 3).
- The *D. rotundata* isolate showed only 62.6-63.8% identity at the nucleotide level with the 3 *D. nummularia* isolates.
- The partial RNA-dependent RNA polymerase (RdRp) amplified from the *D. rotundata* isolate (215 aa) was related to the PepMV homolog gene (68.4% identity).
- The partial RdRp sequence obtained for the 3 *D. nummularia* isolates (217 aa) were associated with the PepMV (68.7% identity) and the CymMV (68.2% identity).
- No obvious symptoms were observed on the positive plants which were also infected with DBV (*D. nummularia*) and YMV (*D. rotundata*) respectively.
- Very few filamentous particles (400-600 nm long) were observed by electron microscopy in the semi-purified virus preparation (Photos 1, 2 and 3).
- No serological relation (Elisa tests) was found with 6 other potexviruses (CymMV, HVX, PAMV, PapMV, PepMV and PVX).
- Mechanical transmission to *Nicotiana benthamiana*, *N. bigelovii* and *D. trifida* was unsuccessful (but YMV was transmitted on *D. trifida*).

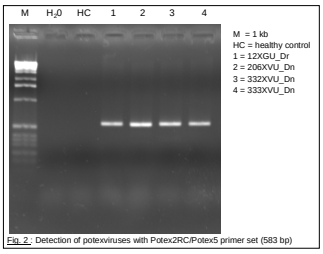
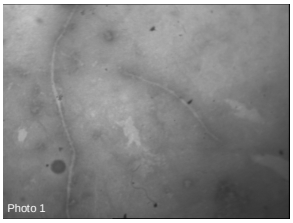


Fig 2 : Detection of potexviruses with Potex2RC/Potex5 primer set (583 bp)



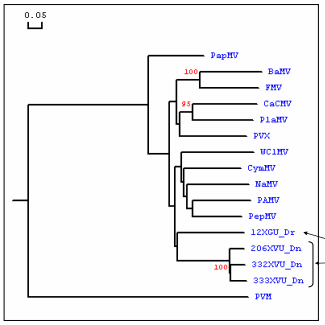
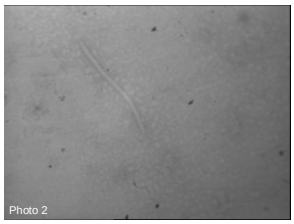
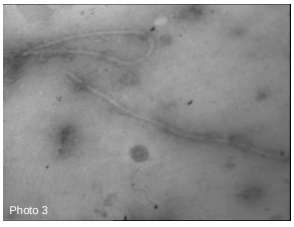


Fig 3 : Phylogenetic relations between sequences of the PCR fragments obtained with the potexvirus primers set (Potex1RC/Potex5 – 696 bp) and the corresponding regions of 11 potexviruses



Two new potexviruses infecting 2 distinct yam species (*D. rotundata* et *D. nummularia*)



Perspectives

- Sequencing of the complete genomes of these two putative members of *Potexvirus* genus.
- Design of primers for the specific and sensitive detection of each potexvirus.
- Screening of new germplasm for these two potexviruses for prevalence studies.
- Monitoring virus elimination (by thermotherapy and/or meristem culture, for example) of infected plants in order to recover potexvirus-free material.

References

Brunt A.A., Jackson G.V.H. and Frison E.A. (eds.). 1989. FAO/IBPGR Technical Guidelines for the Safe Movement of Yam Germplasm. Food and Agriculture Organization of the United Nations, Rome/International Board for Plant Genetic Resources, Rome, 20 p.

Filloux D. and Girard J-C. 2006. Indexing and elimination of viruses infecting yams (*Dioscorea* spp.) for the safe movement of germplasm. Proceedings 14th Triennial Symposium of the International Society for Tropical Root Crops, 20-26 November 2006, Trivandrum, India, 13 p.

Phillips S., Pigott J. d'A. and Brunt A.A., 1986. Further evidence that Dioscorea latent virus is a potexvirus. Annals of Applied Biology 109: 137-145.

van der Vlugt A.A. and Berendsen M., 2002. Development of a general potexvirus detection method, European Journal of Plant Pathology 108: 367-371.

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